Name _____

Investigation 13: Enzyme Activity

Background

Enzymes are the catalysts of biological systems. They speed up chemical reactions in biological systems by lowering the activation energy, the energy needed for molecules to begin reacting with each other. Enzymes do this by forming an enzyme-substrate complex that reduces energy required for the specific reaction to occur. Enzymes have specific shapes and structures that determine their functions. The enzyme's active site is very selective, allowing only certain substances to bind. If the shape of an enzyme is changed in any way, or the protein denatured, then the binding site also changes, thus disrupting enzymatic functions.

Enzymes are fundamental to the survival of any living system and are organized into a number of groups depending on their specific activities. Two common groups are catabolic enzymes ("cata" or "kata-" from the Greek "to break down") — for instance, amylase breaks complex starches into simple sugars — and anabolic enzymes ("a-" or "an" from the Greek "to build up"). (You may know this second word already from stories about athletes who have been caught using anabolic steroids to build muscle.)

Catalytic enzymes, called proteases, break down proteins and are found in many organisms; one example is bromelain, which comes from pineapple and can break down gelatin. Bromelain often is an ingredient in commercial meat marinades. Papain is an enzyme that comes from papaya and is used in some teeth whiteners to break down the bacterial film on teeth. People who are lactose intolerant cannot digest milk sugar (lactose); however, they can take supplements containing lactase, the enzyme they are missing. All of these enzymes hydrolyze large, complex molecules into their simpler components; bromelain and papain break proteins down to amino acids, while lactase breaks lactose down to simpler sugars.

Anabolic enzymes are equally vital to all living systems. One example is ATP synthase, the enzyme that stores cellular energy in ATP by combining ADP and phosphate. Another example is rubisco, an enzyme involved in the anabolic reactions of building sugar molecules in the Calvin cycle of photosynthesis.

To begin this investigation, you will focus on the enzyme peroxidase obtained from a turnip, one of numerous sources of this enzyme. Peroxidase is one of several enzymes that break down peroxide, a toxic metabolic waste product of aerobic respiration. Using peroxidase, you will develop essential skills to examine your own questions about enzyme function.

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Key Vocabulary

Baseline is a universal term for most chemical reactions. In this investigation the term is used to establish a standard for a reaction. Thus, when manipulating components of a reaction (in this case, substrate or enzyme), you have a reference to help understand what occurred in the reaction. The baseline may vary with different scenarios pertinent to the design of the experiment, such as altering the environment in which the reaction occurs. In this scenario, different conditions can be compared, and the effects of changing an environmental variable (e.g., pH) can be determined.

Rate can have more than one applicable definition because this lab has two major options of approach, i.e., using a color palette and/or a spectrophotometer to measure percent of light absorbance. When using a color palette to compare the change in a reaction, you can infer increase, decrease, or no change in the rate; this inference is usually called the relative rate of the reaction. When using a spectrophotometer (or other measuring devices) to measure the actual percent change in light absorbance, the rate is usually referred to as absolute rate of the reaction. In this case, a specific amount of time can be measured, such as 0.083 absorbance/minute.

Part I: Developing a Method for Measuring Peroxidase in Plant Material and Determining a Baseline

Peroxide (such as hydrogen peroxide) is a toxic byproduct of aerobic metabolism. Peroxidase is an enzyme that breaks down these peroxides. It is produced by most cells in their peroxisomes.

The general reaction can be depicted as follows:

Enzyme + Substrate --> Enzyme-Substrate Complex --> Enzyme + Product(s) + ΔG

For this investigation the specific reaction is as follows:

Peroxidase + Hydrogen Peroxide --> Complex --> Peroxidase + Water + Oxygen

$$2H_2O_2 \rightarrow 2H_2O + O_2\,(gas)$$

Notice that the peroxidase is present at the start and end of the reaction. Like all catalysts, enzymes are not consumed by the reactions. To determine the rate of an enzymatic reaction, you must measure a change in the amount of at least one specific substrate or product over time. In a decomposition reaction of peroxide by peroxidase (as noted in the above formula), the easiest molecule to measure would probably be oxygen, a final product. This could be done by measuring the actual volume of oxygen gas released or by using an indicator. In this experiment, an indicator for oxygen will be used. The compound guaiacol has a high affinity for oxygen, and in solution, it binds instantly with oxygen to form tetraguaiacol, which is brownish in color. The greater the amount of oxygen gas produced, the darker brown the solution will become.

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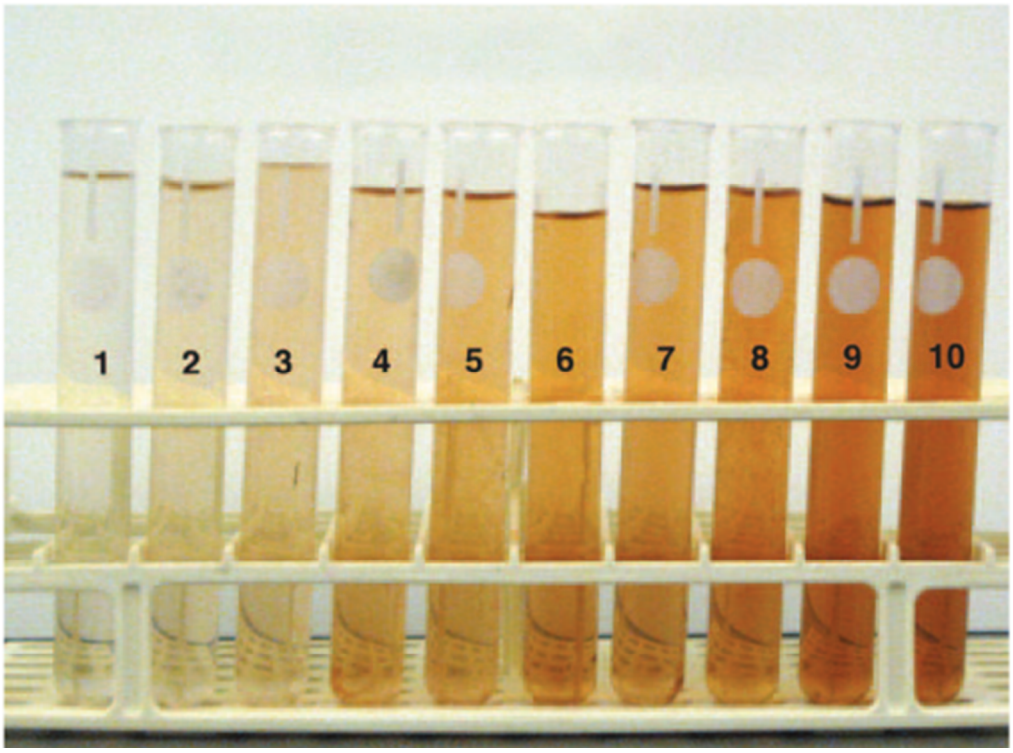
Qualifying color is a difficult task, but a series of dilutions can be made and then combined on a palette, which can represent the relative changes occurring during the reaction. A color palette/chart ranging from 1 to 10 (Figure 1) is sufficient to compare relative amounts of oxygen produced. Alternatively, the color change can be recorded as a change in absorbency using a variety of available meters, such as a spectrophotometer or a probe system.



Turnip Peroxidase Color Chart

Procedure

- Preparing the spectrophotometer
  - Spectrophotometer needs to warm up for about 15 min and set to a wavelength of 470 nm to measure the amount of tetraguaiacol produced
  - Set up zero absorbance using a blank
    - So that any difference in the meter reading with a change in the sample will reflect a difference in oxidized guaiacol concentration
    - Blank contains everything EXCEPT the substrate (hydrogen peroxide)
      - 13.3 mL distilled water
      - 0.2 mL guaiacol
      - 1.5 mL peroxidase (enzyme extract)

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- Determining the baseline
  - Prepare 2 separate test tubes
    - Substrate tube:
      - 7 mL distilled water
      - 0.3 mL of 0.1% hydrogen peroxide
      - 0.2 mL guaiacol
    - Enzyme tube:
      - 6.0 mL distilled water
      - 1.5 mL peroxidase
  - Combine the contents of the two test tubes, cover with Parafilm, invert twice to mix, and carefully pour into cuvette (2/3 full)
    - Note: Timing starts when the two test tubes are combined
      - So wiped down cuvette must be inserted into spectrophotometer within 30 seconds!
    - Absorbance for time 0 will be recorded as 0.0
  - Continue recording absorbance every 30 seconds for 5 minutes in Table 1
  - Graph the absorbance vs time (Figure 1)
  - Use the slope of the line to determine the rate of the reaction
    - Δ Absorbance/minute
    - Show your work!

Results

Table 1: Baseline

Time (min)	Absorbance
0	0.0
0.5	
1	
1.5	
2	
2.5	
3	
3.5	
4	
4.5	
5	

Rate of reaction determined from Figure 1: _____ Δ Abs/min

- Show your work here:

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Part II: Determining the Effect of pH on Enzymatic Activity

Procedure

Each group will be responsible for determining the rate of reaction for a different pH. For your group's particular set up:

- Prepare 2 separate test tubes
  - Substrate tube:
    - 7 mL distilled water
    - 0.3 mL of 0.1% hydrogen peroxide
    - 0.2 mL guaiacol
  - Enzyme tube:
    - 6.0 mL specified pH solution
    - 1.5 mL peroxidase

- WHEN IT IS YOUR GROUP'S TURN TO USE THE SPECTROPHOTOMTER:
  - Combine the contents of the two test tubes, cover with Parafilm, invert twice to mix, and carefully pour into cuvette (2/3 full)
    - Note: Timing starts when the two test tubes are combined
      - So wiped down cuvette must be inserted into spectrophotometer within 30 seconds!
    - Absorbance for time 0 will be recorded as 0.0
  - Continue recording absorbance every 30 seconds for 5 minutes in Table 2
    - Be sure to label your pH

- Graph the absorbance vs time (Figure 2)
- Use the slope of the line to determine the rate of the reaction
  - Show your work!

- Record the rate of reaction for each pH in Table 3 (class data)
- Create a bar graph showing the effect of pH on enzyme activity (Figure 3)
  - X-axis: pH
    - Note: Also include a baseline bar for a comparison
  - Y-axis: Rate (Δ Absorbance/minute)

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Results

Table 2: pH ____

Time (min)	Absorbance
0	0.0
0.5	
1	
1.5	
2	
2.5	
3	
3.5	
4	
4.5	
5	

Rate of reaction determined from Figure 2: _____ Δ Abs/min

- Show your work here:

Table 3: Class Reaction Rates

pH	Rate (Δ Abs/min)
Baseline	
3	
5	
7	
10	

Conclusions:

- Based on the class results, what trends do you notice in terms of pH and reaction rates?

- Explain at least two possible sources of error in your procedure/data collection that could affect the results in this lab

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Part III: What Other Factors Influence the Rates of Enzymatic Reactions?

Pick another factor (other than pH) that you think would influence the rate of enzymatic reactions. Write a detailed, reproducible procedure explaining the steps you would take to test AND analyze the effects of that variable.

Variable Chosen:

Procedure: